NIT-133-06

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Prior Application: K. YASUDA et al
Serial No. 09/666,883
Filed: September 20, 2000

Group Art Unit: 1655
Examiner: B. Forman
For: POLYNUCLEOTIDE SEPARATION METHOD AND APPARATUS THEREFOR

PRELIMINARY AMENDMENT

Commissioner of Patents
Washington, D.C. 20231

Sir:

Prior to examination, please amend the above-identified application as follows:

IN THE SPECIFICATION

Page 1, before the first line of the specification, please insert the sentence:

--This is a continuation application of U.S. Serial No. 09/666,883, filed September 20, 2000, which is a continuation application of U.S. Serial No. 09/522,465, filed on March 9, 2000 (now U.S. Patent No. 6,218,126), which is a continuation application of U.S. Serial No. 09/329,318, filed June 10, 1999 (now U.S. Patent No. 6,093,370). This application is related to U.S. Serial No. 09/790,872, filed on February 23, 2001.

Pages 16 and 17, the paragraph bridging these pages from page 16, line 22 to page 17, line 19, replace the paragraph with:

Convergent light 51 is then irradiated through the objective lens 15 to the target polynucleotide hybridization area 142 where the target polynucleotide 42' is hybridized to the probe 42; the photoabsorbing layer 21 in the area 142 absorbs the convergent light 51 and evolves heat. The heat from the photoabsorbing layer 21 in the area 142 allows the vicinity of the area 142 to increase its temperature up to about 95°C, and hence hydrogen bonds between the probe 42 and the target polynucleotide 42' are dissociated to denature the target polynucleotide 42' along which has been hybridized to the area 142. When the size of an area where the convergent light is converged is smaller than that of a unit target polynucleotide hybridization area, the light axis should be adjusted to ensure that the convergent area is within the target polynucleotide hybridization area. When a unit target polynucleotide hybridization area has a smaller size than the convergent area of the convergent light, individual areas should preferably be arranged in such a manner that gaps between individual target polynucleotide hybridization areas are sufficient and only one area is to be heated by the convergent light. In FIG. 3, only one probe is shown in each target polynucleotide hybridization area to be easy to read, but in practice, a plurality of probes having an identical base sequence are generally immobilized to each area.

Pages 18 and 19, the paragraph bridging these pages from page 18, line 14 to page 19, line 2, replace the paragraph with:

FIG. 4 illustrates a second means for heating a specific area on the substrate 1. The photoabsorbable thin layer 21 is formed on the target polynucleotide hybridization areas in the embodiment of FIG. 3, whereas, in the present embodiment, particles 23 each having photoabsorbing characteristics and have sufficiently small sizes in comparison with those of the target polynucleotide hybridization areas are dispersed and placed on the target polynucleotide hybridization areas. At least one particle should be placed on each area. According to the present embodiment, heat insulating layer 22 is separately provided in each of individual areas and the particles 23 are placed onto the upper surface of the insulating layer 22. The substrate 1 comprises substrate base 13 composed of electrically conductive film 131 and thermally conductive insulating substrate 132 as well as in the embodiment illustrated in FIG. 3.--

IN THE CLAIMS

Cancel claim 1, and add new claims 30-37 as follows:

--30. An apparatus for recovering a target polynucleotide in a cell comprising:

a substrate being disposed in a separation cell, wherein the sample solution containing cells each containing polynucleotides and protein is supplied on a surface of the substrate, a plurality of independent areas are formed on the surface of the substrate and each of a single-stranded oligonucleotide probes each having a specific base sequence is immobilized to each of the areas;

capturing means for capturing each of the cells one by one separately on each of the areas;

means for applying a DC field onto a surface of one area of the areas;

temperature measuring means for measuring a temperature of the surface of the substrate at one area of the areas;

heating or cooling means for heating or cooling the surface of the substrate at the one area of the areas; and

controling means for controling selectively the temperature of the surface of the substrate at the one area on the basis of a temperature information obtained by the temperature measuring means, by controlling the heating or cooling means,

wherein the controling means controls the heating or cooling means so as to heat the surface of the substrate at the one area of the areas to a first predetermined temperature to destroy the cell captured at the one area, to liberate the

polynucleotides and the proteins from the cell captured at the one area, and to denature the polynucleotides liberated from the cell so as to obtain single-stranded polynucleotides, and the controling means controls the heating or cooling means so as to cool a solution which contains no polynucleotide and has a pH value of 4 or lower and with which the sample solution on the substrate is replaced, to a second predetermined temperature to form hybrids between the single-stranded polynucleotides and the single-stranded oligonucleotide probes, so as to capturing single-stranded target polynucleotides;

wherein, after separating the single-stranded polynucleotides and the proteins, whereby the hybrids remain on the one area, by electrophoresis under the DC field applied onto the surface of the one area, based on a charge difference between the single-stranded target polynucleotides and the proteins, in the solution having a value of pH being 4 or lower, by flowing a washing solution into the separation cell, whereby the cells at the areas except for the one area remain on the areas and the hybrids remain on the one area, the washing solution is recovered to recover the proteins liberated from the cell;

wherein, after separating the single-stranded polynucleotides not forming the hybrids, whereby the hybrids

remain on the one area, by electrophoresis under the DC field applied the surface of the one area, by flowing the washing solution into the separation cell, the washing solution is recovered to recover the single-stranded polynucleotides not forming the hybrid;

wherein, after heating the surface of the substrate at the one area of the areas to denature the hybrids at the one area, so as to liberate the single-stranded target polynucleotides into solution, by flowing the washing solution into the separation cell, the washing solution is recovered to recover the single-stranded target polynucleotides liberated from the cell; and

wherein, by changing a position of the one area of the areas, the washing solution is recovered to recover, separately, the proteins, the single-stranded polynucleotides not forming the hybrid, and the single-stranded target polynucleotides, for each of the areas.

--31. An apparatus according to claim 30, wherein the cell is a white blood cell.

--32. An apparatus according to claim 30, wherein the single-stranded target polynucleotide is mRNA.

--33. An apparatus for recovering a target polynucleotide in a cell comprising:

a substrate being disposed in a separation cell, wherein the sample solution containing cells is supplied on a surface of the substrate, and a plurality of independent areas are formed on the surface of the substrate;

capturing means for capturing each of the cells one by one separately on each of the areas;

means for applying a DC field onto a surface of one area of the areas;

temperature measuring means for measuring a temperature of the surface of the substrate at one area of the areas;

heating or cooling means for heating or cooling the surface of the substrate at the one area of the areas;

controling means for controling selectively the temperature of the surface of the substrate at the one area on the basis of a temperature information obtained by the temperature measuring means, by controling the heating or cooling means; and

means for identifying the positions of the areas where the cells to be destroyed are present,

wherein the controling means controls the heating or cooling means so as to heat the surface of the substrate at one of the identified positions to a first predetermined temperature to

destroy the cell captured at the surface of the one of the identified positions, to liberate the polynucleotides and the proteins from the cell captured at the area of the one of the identified positions, and to denature the polynucleotide liberated from the cell so as to obtain a single-stranded polynucleotide, and the controling means controls the heating or cooling means so as to cool a solution which contains no polynucleotide and has a pH value of 4 or lower and with which the sample solution on the substrate is replaced, to a second predetermined temperature to form hybrids between the single-stranded polynucleotides and the single-stranded oligonucleotide probes, so as to capturing single-stranded target polynucleotides;

wherein, after separating the single-stranded polynucleotides and the proteins, whereby the hybrids remain on the area of the one of the identified positions, by electrophoresis under the DC field applied onto the surface of the area of the one of the identified positions, based on a charge difference between the single-stranded target polynucleotides and the proteins, in the solution having a value of pH being 4 or lower, by flowing a washing solution into the separation cell, whereby the cells at the areas except for the area of the one of the identified positions remain on the areas and the hybrids remain on the area of the

one of the identified positions the washing solution is recovered to recover the proteins liberated from the cell; wherein, after separating the single-stranded polynucleotides not forming the hybrids, whereby the hybrids remain on the area of the one of the identified positions, by electrophoresis under the DC field applied onto the surface of the area of the one of the identified positions, by flowing the washing solution into the separation cell, the washing solution is recovered to recover the single-stranded polynucleotides not forming the hybrid;

wherein, after heating the surface of the substrate at the one of the identified positions to denature the hybrids at the area of the one of the identified positions, so as to liberate the single-stranded target polynucleotides into solution, by flowing the washing solution into the separation cell, the washing solution is recovered to recover the single-stranded target polynucleotides liberated from the cell; and

wherein, by changing a position of the identified positions, the washing solution is recovered to recover, separately, the proteins, the single-stranded polynucleotides not forming the hybrid, and the single-stranded target polynucleotide, for each of the identified positions.

--34. An apparatus according to claim 33, wherein the cell is a white blood cell.

--35. An apparatus according to claim 33, wherein the single-stranded target polynucleotide is mRNA.--

REMARKS

Examination is requested.

Respectfully submitted,

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MARKED UP VERSION OF REPLACED PARAGRAPH(S) OF THE SPECIFICATION

Pages 16 and 17, the paragraph bridging these pages from page 16, line 22 to page 17, line 19, replace the paragraph with:

[Divergent] Convergent light 51 is then irradiated through the objective lens 15 to the target polynucleotide hybridization area 142 where the target polynucleotide 42' is hybridized to the probe 42; the photoabsorbing layer 21 in the area 142 absorbs the convergent light 51 and evolves heat. The heat from the photoabsorbing layer 21 in the area 142 allows the vicinity of the area 142 to increase its temperature up to about 95°C, and hence hydrogen bonds between the probe 42 and the target polynucleotide 42' are dissociated to denature the target polynucleotide 42' along which has been hybridized to the area 142. When the size of an area where the convergent light is converged is smaller than that of a unit target polynucleotide hybridization area, the light axis should be adjusted to ensure that the convergent area is within the target polynucleotide hybridization area. When a unit target polynucleotide hybridization area has a smaller size than the [divergent] convergent area of the [divergent] convergent light, individual areas should preferably be arranged in such a manner that gaps between individual target polynucleotide hybridization areas are sufficient and only one area is to be

heated by the convergent light. In FIG. 3, only one probe is shown in each target polynucleotide hybridization area to be easy to read, but in practice, a plurality of probes having an identical base sequence are generally immobilized to each area.

Pages 18 and 19, the paragraph bridging these pages from page 18, line 14 to page 19, line 2, replace the paragraph with:

FIG. 4 illustrates a second means for heating a specific area on the substrate 1. The [photoabsorvable] photoabsorbable thin layer 21 is formed on the target polynucleotide hybridization areas in the embodiment of FIG. 3, whereas, in the present embodiment, particles 23 each having photoabsorbing characteristics and have sufficiently small sizes in comparison with those of the target polynucleotide hybridization areas are dispersed and placed on the target polynucleotide hybridization areas. At least one particle should be placed on each area. According to the present embodiment, heat insulating layer 22 is separately provided in each of individual areas and the particles 23 are placed onto the upper surface of the insulating layer 22. The substrate 1 comprises substrate base 13 composed of electrically conductive film 131 and thermally conductive insulating substrate 132 as well as in the embodiment illustrated in FIG. 3.--